

# Effect of Internal Bleaching on Discoloration Caused by 4 Different Materials

Shiva Shojaeian , Nazanin Zargar , Maryam Amiri 

<sup>a</sup>Assistant Professor, Dept of Endodontics, School of Dentistry, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

<sup>b</sup>Associate Professor, Dept of Endodontics, School of Dentistry, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

<sup>c</sup>Endodontist, School of Dentistry, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

Correspondence to Maryam Amiri (email: drmrym91@gmail.com).

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**Objectives** In endodontics, mineral trioxide aggregate (MTA) and calcium-enriched mixture (CEM cement) are commonly used bioceramic materials. Regenerative endodontics often involves the use of double or triple antibiotic pastes (DAP or TAP), which are effective but may cause discoloration. This study aims to examine the effectiveness of internal bleaching in addressing discoloration caused by 4 different materials.

**Methods** This in vitro study was conducted on 40 single-rooted anterior and premolar human teeth that did not have caries, restorations, obvious discoloration, or fractures and were extracted due to periodontal disease or orthodontic treatment. The teeth were randomly assigned to 4 groups (n=10). After root canal instrumentation, the baseline color of the specimens was measured using a spectrophotometer. After applying the aforementioned materials in the canals, access cavities were sealed with glass ionomer (GI), and the color of the specimens was measured again after 1 month to calculate color change ( $\Delta E$ ). Internal bleaching was performed with 35% hydrogen peroxide 3 times within 3 weeks, and values were calculated at each time point. Data were analyzed using paired t-tests and 1-way analysis of variance (ANOVA).

**Results** Clinically noticeable discoloration ( $\Delta E > 3.7$ ) occurred in the MTA Angelus ( $\Delta E = 4.88$ ) and TAP ( $\Delta E = 17.3$ ) groups. The  $\Delta E$  of the TAP group was significantly higher than that of all other groups ( $P < 0.05$ ). The discoloration was borderline detectable in the CEM group ( $\Delta E = 3.89$ ) and clinically undetectable in the DAP group ( $\Delta E = 2.02$ ). Discoloration significantly improved after 3 rounds of bleaching in the TAP group ( $P < 0.05$ ), as well as after 2 rounds of bleaching in the MTA ( $P < 0.05$ ) and CEM ( $P < 0.05$ ) groups. In all groups,  $\Delta E$  increased after each round of bleaching.

**Conclusion** The CEM cement may yield better results with lower discoloration compared to MTA and exhibit a more favorable response to internal bleaching. For canal disinfection using antibiotic paste, DAP is a good alternative to TAP.

**Keywords** Calcium-Enriched Mixture Cement; Mineral Trioxide Aggregate; Tooth Bleaching; Tooth Discoloration

## Introduction

Tooth discoloration refers to any change in the hue, color, or translucency of teeth caused by restorations, restorative materials, root canal therapy medications (local/systemic), pulp necrosis, aging, or hemorrhage. Discoloration may occur due to internal stains within the tooth structure or external stains on the tooth surface.<sup>1, 2</sup> Discoloration resulting from the application of endodontic materials is a common occurrence that can compromise the esthetic appearance of endodontically treated teeth or after regenerative endodontic treatment.<sup>3, 4</sup>

Mineral trioxide aggregate (MTA) is a dental cement widely used in endodontics for applications such as apexification, perforation repair, root-end filling, and root revascularization.<sup>5, 6</sup> Calcium-enriched mixture (CEM) cement has similar applications to MTA and can stimulate stem cell proliferation and hydroxyapatite crystal formation. Additionally, CEM cement possesses antibacterial and antifungal properties, which sets it apart from MTA.<sup>5, 7</sup>

Triple antibiotic paste (TAP) is a mixture of metronidazole, ciprofloxacin, and minocycline in a 1:1:1 ratio.<sup>8</sup> It is effective in eliminating pathogenic microorganisms from the root canal system<sup>9-12</sup> and creates a nearly sterile environment conducive to pulp tissue revitalization or

regeneration in the root canals of necrotic immature open-apex teeth.<sup>11, 13</sup> However, minocycline in its composition can lead to tooth discoloration.<sup>14, 15</sup> To address this issue, double antibiotic paste (DAP) was introduced, omitting minocycline from its composition.<sup>16</sup>

Despite the advantages of these dental materials, various levels of tooth discoloration have been reported following the use of MTA, CEM cement, and antibiotic pastes.<sup>9, 17, 18</sup> Bleaching is a treatment method involving the application of a chemical oxidative agent that enhances light absorption or reflection by the tooth structure, resulting in a whiter appearance of teeth.<sup>19, 20</sup> Internal tooth bleaching, on the other hand, is a procedure used to treat discolored teeth that have previously undergone endodontic treatment. It is a minimally invasive, conservative, relatively simple, effective, and cost-effective method.<sup>21, 22</sup>

Considering that the use of these materials is inevitable and that we may not yet have better alternatives, this study aimed to assess the discoloration caused by 4 common materials in endodontic treatment (CEM cement, MTA, DAP, and TAP). Additionally, we examined the effect of internal bleaching on this discoloration.

## Methods and Materials

This in vitro experimental study was approved by the

Ethics Committee of Shahid Beheshti University of Medical Sciences (IR.SBMU.RIDS.REC.1395.279).

The specimens included 40 single-rooted anterior and premolar healthy teeth that were extracted due to hopeless periodontal prognosis or orthodontic treatment. Inclusion criteria were normal tooth color, single-rooted, and normal anatomy, and no coronal or radicular caries. Exclusion criteria included the presence of any cracks, fractures, metamorphosis calcification, or physiological discoloration.

#### Sample Size

The sample size was calculated using the formula below, resulting in a minimum of 8 specimens in each group based on a previous study.<sup>23</sup> The assumptions made were  $\alpha=0.05$ ,  $d=0.05$ , 95% CI, and discoloration caused by MTA to be  $\pm 3.72$  vs  $\pm 1.87$  in the control group.

$$n^* = \frac{(Z_{1-\frac{\alpha}{2}} + Z_{1-\beta})^2 (S_1^2 + S_2^2)}{(\bar{X}_1 - \bar{X}_2)^2}$$

The sample size was calculated based on Jang et al<sup>23</sup>, and kirchoff et al, studies. The difference between the  $\Delta E$  values of the 2 groups was suggested to be 17-8.3=8.7, with  $SD1=4.56$  and  $SD2=3.7$ . To detect this difference between the groups with  $\alpha=5\%$  and  $\beta=0.2$ , at least  $n=5$  samples were needed. However, since we have 4 groups, we adjusted it to at least 9 samples for each group.

#### Sample Preparation

The teeth were disinfected by immersion in 1.5% NaOCl (Nikdarman, Iran) for 12 hours and then stored in saline until the experiment.

The root canals were instrumented, and the access cavities were sealed with glass ionomer (GI; GC, Japan). The baseline color of the teeth was then measured using a spectrophotometer (MHT, Italy) in the CIE  $L^*a^*b^*$  color space. To facilitate this measurement, a mold measuring 10 x 4 cm was fabricated by using putty impression material (Speedex, Coltene, Switzerland). This mold allowed for easy adjustment of the spectrophotometer's probe tip over the putty mold, with the tooth placed at the center of the mold. To measure the tooth color, a circle with a 6-mm diameter, tangent to the cemento-enamel junction (CEJ), was drawn.

The teeth were then randomly divided into 4 groups ( $n=10$ ): MTA, CEM cement, DAP, and TAP.

#### Mineral Trioxide Aggregate and Calcium-Enriched Mixture Cement Groups

The root canals in these groups were instrumented and filled with gutta-percha (Meta-Biomed, South Korea) up to 3 mm below the CEJ. In the MTA group, MTA (Angelus, Brazil) was applied in a 3-mm thickness layer, while in the CEM group, CEM cement (Bionique Dent, Iran) was applied in a 3-mm thickness layer. A moist cotton pellet was placed over the materials for setting, and the cavity was sealed with a layer of Cavit temporary restorative

material (3M, USA), and it was covered by GI (GC, Japan). After 24 hours, the cavity was restored.<sup>23</sup>

#### Double Antibiotic Paste and Triple Antibiotic Paste Groups

In the TAP group, 300 mg of each metronidazole (Jalinous, Iran), minocycline (Jalinous, Iran), and ciprofloxacin (Jalinous, Iran) were mixed in a 1:1:1 ratio. The obtained powder was mixed with 0.3 mL of distilled water to achieve a paste consistency.<sup>8</sup> The triple antibiotic paste was delivered into the root canals using a Lentulo spiral, and the pulp chamber was cleaned with a wet cotton pellet. Then, the access cavity was sealed with GI.

In the DAP group, equal amounts of metronidazole and ciprofloxacin (300 mg each) were mixed, and the rest of the procedure was carried out as in the TAP group.

All teeth were then placed in test tubes containing saline, and their coronal discoloration was quantified using a spectrophotometer after 1 month. The color change between the initial measurement and the measurement after 1 month was calculated (primary  $\Delta E$ ).

#### Internal Bleaching Procedures

Three bleaching sessions were planned. After 1 month of storage in saline, the pulp chamber was completely cleaned with ultrasonic and dried. In the DAP and TAP groups, the intra-canal medicaments were removed using 5 mL of 2.5% sodium hypochlorite and saline with a 27-gauge side-vented needle that was inserted 1-2 mm from the working length.<sup>15</sup>

Next, 35% hydrogen peroxide bleaching gel (Opalescence Endo, Ultradent, USA) was injected into the pulp chamber<sup>24</sup>, and GI was applied over it to seal the access cavity. After 1 week, the color of each tooth was measured ( $\Delta E1$ ). Then, fresh bleaching gel was injected again into the pulp chamber, and GI was applied over it (second round of bleaching). After 1 week, the color of each tooth was measured again ( $\Delta E2$ ). This process was repeated for the third time ( $\Delta E3$ ).

In the CEM and MTA groups, the GI was removed, and the pulp chamber was cleaned, but the MTA and CEM cement were not removed. Bleaching gel was then injected into the pulp chamber, and composite resin was applied over it. The bleaching procedure was performed as described for the DAP and TAP groups. Color changes were calculated at each step of bleaching by comparing the color of the tooth crown after bleaching with the initial discolored crown 1 month after the materials were delivered.

The  $L^*$ ,  $a^*$ , and  $b^*$  color parameters were measured for each tooth at each time of color assessment, and the color change ( $\Delta E$ ) was calculated using the following formula:

$$\Delta E = \sqrt{(L_2 - L_1)^2 + (a_2 - a_1)^2 + (b_2 - b_1)^2}$$

where  $L^*$  indicates lightness and ranges from 0 (very dark) to 100 (very light),  $a^*$  indicates redness-greenness (positive values determine the amount of red, and negative values determine the amount of green), and  $b^*$  indicates yellowness-blueness (positive values determine the amount

of yellow, and negative values determine the amount of blue). A  $\Delta E$  of 3.7 was considered a visually detectable color change.<sup>25, 26</sup>

- $\Delta E_p$ : Difference between the initial teeth color and the color after 1 month of placing the material.
- $\Delta E1$ : Difference between the tooth color after 1 month of placing the materials and the color after the first round of bleaching.
- $\Delta E2$ : Difference between the tooth color after 1 month of placing the materials and the color after the second round of bleaching.
- $\Delta E3$ : Difference between the tooth color after 1 month of placing the materials and the color after the third round of bleaching.

The primary  $\Delta E$  (the amount of darkening after 1 month of placing the material) is compared with  $\Delta E1$ ,  $\Delta E2$ , and  $\Delta E3$  (the amount of whitening after each bleaching). If the primary  $\Delta E$  is equal to or less than  $\Delta E1$ ,  $\Delta E2$ , or  $\Delta E3$ , it indicates that the tooth color has been improved as much as the original tooth color or even better. However, at each stage of bleaching, if the initial  $\Delta E$  is greater than  $\Delta E1$ ,  $\Delta E2$ , or  $\Delta E3$ , it shows that the color of the tooth has not

changed as much as the primary discoloration and has not returned to its original color.

#### Statistical Analysis

The data were analyzed using SPSS version 22 (SPSS Inc, IL, USA). The normal distribution of data was evaluated by the Kolmogorov-Smirnov test. Accordingly, data were analyzed by paired t-test for intra-group comparisons and 1-way analysis of variance (ANOVA) for inter-group comparisons at a significance level of  $P < 0.05$ .

#### Results

The 4 groups showed no significant difference in color parameters before the intervention ( $P > 0.05$ ).

Primary  $\Delta E$  at 1 Month after the Application of MTA, CEM, TAP, and DAP

Tooth discoloration occurred in the MTA and TAP groups, and  $\Delta E$  exceeded the clinically acceptable threshold ( $\Delta E > 3.7$ ).  $\Delta E$  in the CEM group was borderline detectable ( $\Delta E = 3.9$ ), while in the DAP group, it was below this threshold ( $\Delta E < 3.7$ ; Table 1).

**Table 1-** Mean and standard deviation of  $\Delta a$ ,  $\Delta b$ ,  $\Delta L$ , and  $\Delta E$  before and after three rounds of bleaching in the four groups

Groups		$\Delta L$	$\Delta a$	$\Delta b$	$\Delta E$
		Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)
MTA	Primary	-2 (0.4)	1.8 (0.3)	-2.8 (1)	4.88 (2.37)
	1	4.2 (1.4)	-0.4 (0.1)	-0.5 (0)	5.48 (2.27)
	2	6.8 (2.3)	-1.3 (0.2)	-1.5 (0.2)	8.16 (2.79)
	3	6.7 (2.1)	-1.2 (0.2)	-3.9 (0.6)	8.21 (2.23)
CEM	Primary	0.4 (0)	3.3 (0.6)	-0.6 (0)	3.89 (0.933)
	1	3.4 (1)	-1.6 (0.1)	-2 (0.4)	4.58 (3.19)
	2	5.1 (1.4)	-2.4 (0.4)	-3.7 (1.1)	7.2 (3.74)
	3	4.8 (1.6)	-2.3 (0.9)	-5.1 (2.6)	7.7 (3.99)
DAP	Primary	0.4 (0)	-0.5 (0)	0.2 (0)	2.02 (0.967)
	1	3 (1.4)	-1.9 (0.6)	3.1 (0.5)	5.63 (2.47)
	2	5.5 (3.1)	-2.6 (1)	5.9 (1.4)	8.36 (2.3)
	3	5.5 (2.7)	-2.4 (0.9)	-6.7 (2.2)	9.14 (2.24)
TAP	Primary	-15.4 (5.5)	3.8 (1.2)	6.4 (1.2)	17.3 (3.38)
	1	5.6 (3.1)	5.6 (2.1)	-6 (1.8)	10.028 (1.533)
	2	9.9 (+4.6)	6.4 (3.2)	-8.6 (-3.4)	14.228 (1.937)
	3	11.9 (4.8)	3.2 (1.1)	-11 (4.6)	16.800 (2.115)

SD: Standard deviation; Primary: Primary discoloration; 1: After the first round of bleaching; 2: After the second round of bleaching; 3: After the third round of bleaching.

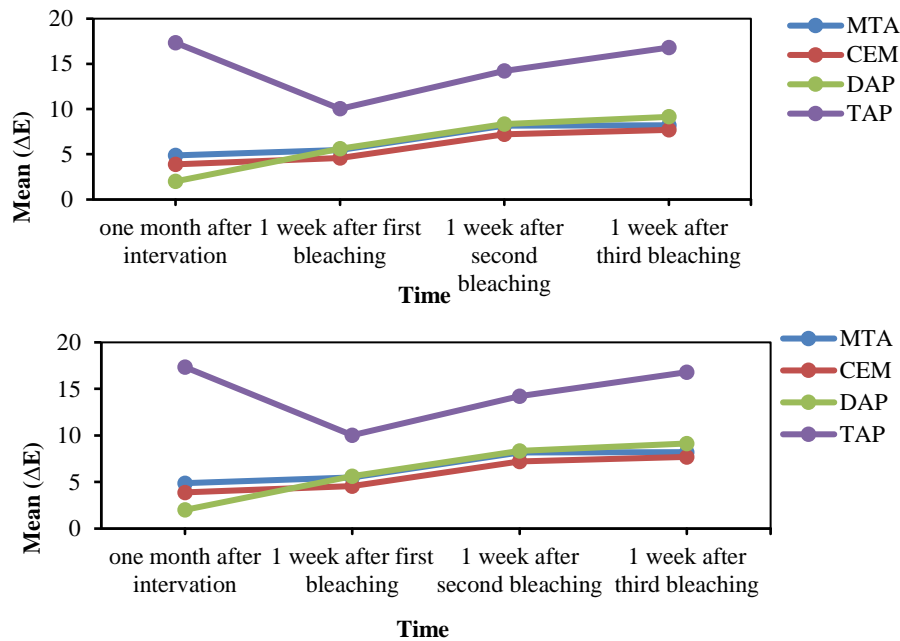
$\Delta E_p$ : Primary  $\Delta E$

Comparison of  $\Delta E$  between the 4 groups showed a significantly higher  $\Delta E$  in the TAP group ( $P < 0.001$ ); however, the difference between the other groups was not significant.

#### $\Delta E$ after Bleaching Steps

The improvement in crown discolorations after different

times of bleaching was compared in each group using paired t-tests. In all 4 groups,  $\Delta E$  increased after each round of bleaching ( $P < 0.05$ ; Figure 1).



**Figure 1:** Deviation of  $\Delta E$  before and after three rounds of bleaching in the four groups

In the MTA group,  $\Delta E_2$  and  $\Delta E_3$  were significantly higher than the primary  $\Delta E$  ( $P=0.023$  and  $P=0.02$ , respectively) and  $\Delta E_1$  ( $P<0.001$  and  $P<0.001$ , respectively). However, the difference between  $\Delta E_3$  and  $\Delta E_2$ , as well as between  $\Delta E_1$  and  $\Delta E$ , was not significant.

In the CEM cement group,  $\Delta E_1$  was insignificantly higher than the primary  $\Delta E$  ( $P=0.618$ ).  $\Delta E_2$  and  $\Delta E_3$  were significantly higher than the primary  $\Delta E$  ( $P=0.023$  and  $P=0.017$ , respectively) and  $\Delta E_1$  ( $P<0.001$  and  $P=0.001$ ). However, the difference between  $\Delta E_3$  and  $\Delta E_2$  was not significant ( $P=0.222$ ).

In the DAP group,  $\Delta E_1$  and  $\Delta E_2$  were significantly higher than the primary  $\Delta E$  ( $P=0.003$  and  $P<0.001$ , respectively).  $\Delta E_3$  was also significantly higher than the primary  $\Delta E$  ( $P=0.017$ ) and  $\Delta E_1$  ( $P<0.001$ ). However, the difference between  $\Delta E_3$  and  $\Delta E_2$  was not significant ( $P=0.668$ ).

In the TAP group,  $\Delta E_1$  was significantly lower than the primary  $\Delta E$  ( $P<0.001$ ).  $\Delta E_2$  and  $\Delta E_3$  were insignificantly lower than the primary  $\Delta E$ .  $\Delta E_2$  and  $\Delta E_3$  were significantly higher than  $\Delta E_1$  ( $P=0.004$  and  $P<0.001$ , respectively).  $\Delta E_3$  was significantly higher than  $\Delta E_2$  ( $P<0.001$ ).

One-way ANOVA revealed a significant difference in the primary  $\Delta E$  before bleaching between the groups ( $P<0.001$ ). Also,  $\Delta E_1$  ( $P<0.001$ ),  $\Delta E_2$  ( $P<0.001$ ), and  $\Delta E_3$  ( $P<0.001$ ) were significantly different between the 4 groups.

In the MTA group,  $\Delta E_2$  and  $\Delta E_3$  were significantly higher than the primary  $\Delta E$  ( $P=0.023$  and  $P=0.02$ , respectively) and  $\Delta E_1$  ( $P<0.001$  and  $P<0.001$ , respectively). However, the difference between  $\Delta E_3$  and  $\Delta E_2$ , as well as between  $\Delta E_1$  and  $\Delta E$ , was not significant.

In the CEM cement group,  $\Delta E_1$  was insignificantly higher

than the primary  $\Delta E$  ( $P=0.618$ ).  $\Delta E_2$  and  $\Delta E_3$  were significantly higher than the primary  $\Delta E$  ( $P=0.023$  and  $P=0.017$ , respectively) and  $\Delta E_1$  ( $P<0.001$  and  $P=0.001$ ). However, the difference between  $\Delta E_3$  and  $\Delta E_2$  was not significant ( $P=0.222$ ).

In the DAP group,  $\Delta E_1$  and  $\Delta E_2$  were significantly higher than the primary  $\Delta E$  ( $P=0.003$  and  $P<0.001$ , respectively).  $\Delta E_3$  was also significantly higher than the primary  $\Delta E$  ( $P=0.017$ ) and  $\Delta E_1$  ( $P<0.001$ ). However, the difference between  $\Delta E_3$  and  $\Delta E_2$  was not significant ( $P=0.668$ ).

In the TAP group,  $\Delta E_1$  was significantly lower than the primary  $\Delta E$  ( $P<0.001$ ).  $\Delta E_2$  and  $\Delta E_3$  were insignificantly lower than the primary  $\Delta E$ .  $\Delta E_2$  and  $\Delta E_3$  were significantly higher than  $\Delta E_1$  ( $P=0.004$  and  $P<0.001$ , respectively).  $\Delta E_3$  was significantly higher than  $\Delta E_2$  ( $P<0.001$ ).

One-way ANOVA revealed a significant difference in the primary  $\Delta E$  before bleaching between the groups ( $P<0.001$ ). Also,  $\Delta E_1$  ( $P<0.001$ ),  $\Delta E_2$  ( $P<0.001$ ), and  $\Delta E_3$  ( $P<0.001$ ) were significantly different between the 4 groups.

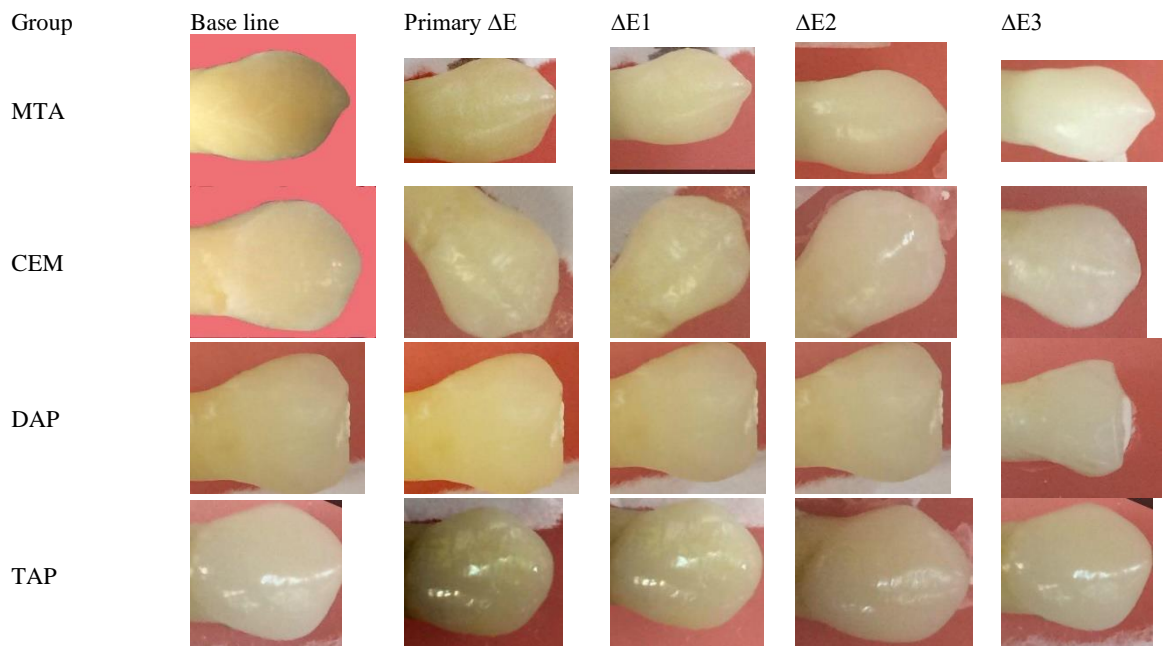
## Discussion

In this study, the response to internal bleaching after discoloration caused by 2 antibiotic pastes and 2 calcium silicate-based materials was evaluated.

The present results show that internal bleaching with 35% hydrogen peroxide improved coronal tooth color in all groups (Figure 2). Similar to our study, Yasa et al<sup>27</sup> found that bleaching teeth dyed with TAP yielded acceptable results with 35% hydrogen peroxide. However, other studies reported different results. Padilla et al<sup>28</sup> showed that after the use of TAP for regeneration, H<sub>2</sub>O<sub>2</sub> alone

could not lead to acceptable results, so they used a low concentration of H<sub>2</sub>O<sub>2</sub> in combination with sodium perborate. Iriboz et al<sup>29</sup> used ultrasound to activate sodium

perborate and showed that the results of internal bleaching were not favorable. In this study, a high concentration of H<sub>2</sub>O<sub>2</sub> (35%) was used to achieve better results.



**Figure 2: Representative specimens from each of the four groups to show their natural color, and color change after the application of materials and after different rounds of bleaching**

One month after the application of the materials, the L\* parameter decreased, and the tooth crowns became darker in all groups except the DAP group. In the MTA and CEM groups, the a\* parameter was positive, indicating a shift toward red, and the b\* parameter was negative, indicating a shift toward blue. In the TAP and DAP groups, the a\* parameter was negative, indicating a shift toward green, and the b\* parameter was positive, indicating a shift toward yellow.

In the TAP group, discoloration significantly improved after each round of bleaching, but it was only after the third round that tooth discoloration returned to the original tooth color (the difference between  $\Delta E_3$  and  $\Delta E_p$  was insignificant). Improvement was noticed in all 3 color coordinates of L\*, a\*, and b\*. The L\* parameter increased after each round of bleaching, causing the crowns to become lighter. Additionally, the a\* parameter increased, shifting the color toward red, while the b\* parameter decreased.

In the DAP group,  $\Delta E$  was below the perceptible threshold, and after 1 round of bleaching,  $\Delta E_1$  was significantly higher than the primary  $\Delta E$ .  $\Delta L$  also increased, indicating lightening of tooth color compared to the primary color. Consistent with the present findings, Kirchoff et al<sup>25</sup> revealed that discoloration caused by TAP was corrected by bleaching with sodium perborate, and after 3 rounds of bleaching, color correction and an increase in the L\* parameter occurred.

In the present study,  $\Delta E_1$  in the MTA and CEM groups was

slightly, but insignificantly, higher than the primary  $\Delta E$ . Therefore, the tooth discoloration returned to the original color after 1 round of bleaching. Bleaching increased the L\* parameter and shifted the a\* parameter toward green; however, the change in the b\* parameter was not desirable, as it shifted toward blue.

The results revealed that TAP exhibited significant discoloration after 1 month ( $\Delta E=17.3$ ). The greater color change observed with TAP can be attributed to the presence of minocycline in its composition. It is believed that the color change caused by the tetracycline family, including minocycline, results from a photo-initiated reaction.<sup>30</sup>

In a study conducted by Berkhoff et al<sup>31</sup>, it was found that 88% of the TAP remained in the dentin of the root canal wall at a depth of at least 350  $\mu m$ , even after irrigation with various techniques. This finding indicates that TAP has a high capacity to penetrate dentinal tubules, which may be the reason for the higher staining values observed when using TAP in the study.

Due to the potential risk of tooth discoloration caused by minocycline, it is suggested to use DAP instead of TAP or replace minocycline with another antibiotic in the composition of TAP.<sup>25</sup> In a study by F. K uc ukenci et al<sup>32</sup>, TAP was compared with alternatives to minocycline, such as amoxicillin, cefaclor, doxycycline, and DAP. They found that all groups showed noticeable color differences except for the DAP and control groups, which is consistent with the findings of this study.

Consistent with the current findings, Arman et al found no significant difference in the discoloration caused by the tooth-colored formulations of MTA and CEM cement.<sup>33</sup> However, Rouhani et al<sup>34</sup> reported significantly higher tooth discoloration caused by MTA compared to CEM cement. In our study, discoloration caused by MTA was slightly, but not significantly, higher than that caused by CEM cement. The greater discoloration caused by MTA is attributed to the presence of iron and manganese in its formulation.<sup>34</sup> Berger et al<sup>35</sup> suggested that the combination of bismuth oxide with other chemical compounds is the main cause of tooth discoloration by MTA. Additionally, CEM cement contains lower amounts of Al<sub>2</sub>O<sub>3</sub>, MgO, and FeO compared to MTA, and bismuth oxide is not present in its composition.<sup>36</sup>

The staining levels caused by calcium silicate materials can vary, and their response to internal bleaching differs depending on their ingredients.<sup>37</sup> Comparative studies between CEM cement and other materials are limited, making our study innovative in this regard.

It is important to note that in vitro studies cannot fully replicate the complex interactions in the oral environment, including oral functions, food intake, host defense, saliva,

and variations in temperature and pH. Therefore, caution should be exercised when generalizing these results to clinical practice. Further clinical studies are needed to validate the findings of this research.

## Conclusion

Within the limitations of this in vitro study, the results showed that TAP caused the highest discoloration, which was corrected by 3 rounds of bleaching. The double antibiotic paste did not cause significant discoloration. The discoloration caused by MTA was greater than CEM, and both were corrected after 1 round of bleaching.

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## Conflict of Interest

No Conflict of Interest Declared ■

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